Microglial activation and TNFα production mediate altered CNS excitability following peripheral inflammation

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Peripheral inflammation leads to a number of centrally mediated physiological and behavioral changes. The underlying mechanisms and the signaling pathways involved in these phenomena are not yet well understood. We hypothesized that peripheral inflammation leads to increased neuronal excitability arising from a CNS immune response. We induced inflammation in the gut by intracolonic administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) to adult male rats. To examine the excitability of the brain *in vivo*, we administered pentylenetetrazole (PTZ; a GABAergic antagonist) intravenously to evoke clonic seizures. Rats treated with TNBS showed increased susceptibility to PTZ seizures that was strongly correlated with the severity and progression of intestinal inflammation. *In vitro* hippocampal slices from inflamed, TNBS-treated rats showed increased spontaneous interictal burst firing following application of 4-aminopyridine, indicating increased intrinsic excitability. The TNBS-treated rats exhibited a marked, reversible inflammatory response within the hippocampus, characterized by microglial activation and increases in tumor necrosis factor α (TNFα) levels. Central antagonism of TNFα using a monoclonal antibody or inhibition of microglial activation by i.c.v. injection of minocycline prevented the increase in seizure susceptibility. Moreover, i.c.v. infusion of TNFα in untreated rats for 4 days also increased seizure susceptibility and thus mimicked the changes in seizure threshold observed with intestinal inflammation. Our finding of a microglia-dependent TNFα-mediated increase in CNS excitability provides insight into potential mechanisms underlying the disparate neurological and behavioral changes associated with chronic inflammation.

4-aminopyridine | cytokine | pentylenetetrazole | seizure | colitis

Peripheral inflammation is associated with the appearance of an inflammatory response in the brain similar to that generated in the periphery. This is characterized by activation of microglia and increases in proinflammatory cytokines, including tumor necrosis factor alpha (TNFα), interleukin (IL)-1β, and IL-6 (reviewed in ref. 1). Despite evidence that cytokines can directly influence synaptic function and neuronal properties *in vitro* (2–7) as well as alter transmitter biosynthetic pathways (1, 8, 9), the underlying mechanisms that link the peripheral inflammation, the central neuroimmune response, and CNS function are poorly understood.

CNS inflammation has long been recognized as an important factor in seizure pathophysiology (10). In contrast, the possibility that peripheral inflammation may likewise contribute to a hyperexcitable state has received considerably less attention. This is important to know, as an understanding of the relationship between peripheral inflammation and neuronal excitability may help explain some of the underlying comorbidities that are reported to exist between peripheral inflammatory disease and seizure disorders, including epilepsy (11, 12).

Here we have tested the hypothesis that peripheral inflammation will alter neuronal excitability in the hippocampus, a brain area important in seizure generation and propagation. We have used a model of peripheral inflammation induced by intracolonic administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS) to initiate a T helper-1 cell-mediated model of inflammatory bowel disease; in its acute phase, this is associated with immune system activation and causes a localized inflammatory colitis (13–16). This has enabled us to evaluate brain excitability both *in vivo* and *in vitro* over a 10 day period of acute and resolving inflammation and identify the roles of microglia and cytokines in these responses. We have identified a microglia-dependent TNFα-mediated increase in CNS excitability that may provide a better understanding of how diverse types of peripheral inflammation might have a common presentation, and also provide insight into comorbidities resulting from altered CNS function in peripheral inflammatory diseases.

Results

Peripheral Inflammation Increases Seizure Susceptibility. In animals treated with TNBS, the peak of inflammation in the gut is observed 4 days after treatment, and slowly resolves over the next 10 days (13, 17, 18). We determined the susceptibility to the convulsant pentylenetetrazole (PTZ) in TNBS- and saline-treated rats. Rats (four to five at each time point) underwent PTZ seizure-susceptibility testing at 2, 4, or 10 days after colonic treatment. These time points were chosen to reflect the initial inflammation (2 days), the height of inflammation (4 days), and the resolving phase of inflammation (10 days) after TNBS treatment. Rats with intestinal inflammation had significantly enhanced seizure susceptibility when compared with controls, with the maximal increased susceptibility observed 4 days after inflammation induction ($P < 0.001$; Fig. 1*A*), corresponding to the peak severity of inflammation.

To determine if there was a correlation between the severity of the inflammation and seizure susceptibility, additional rats underwent induction of a milder intestinal inflammation. When bowel damage scores (a measure of the degree of the inflammation) and seizure susceptibility scores were evaluated 4 days later, we detected a strong and statistically significant correlation between the bowel damage and seizure susceptibility scores ($r = 0.689$, $P < 0.01$; Fig. 1*B*).

Peripheral Inflammation Increases Hippocampal Slice Excitability. If the increased CNS excitability during peripheral inflammation is due to intrinsic changes in network excitability, then brain slices obtained from TNBS-treated rats should be more prone to excitation. To test for this, we perfused slices with 100 μM

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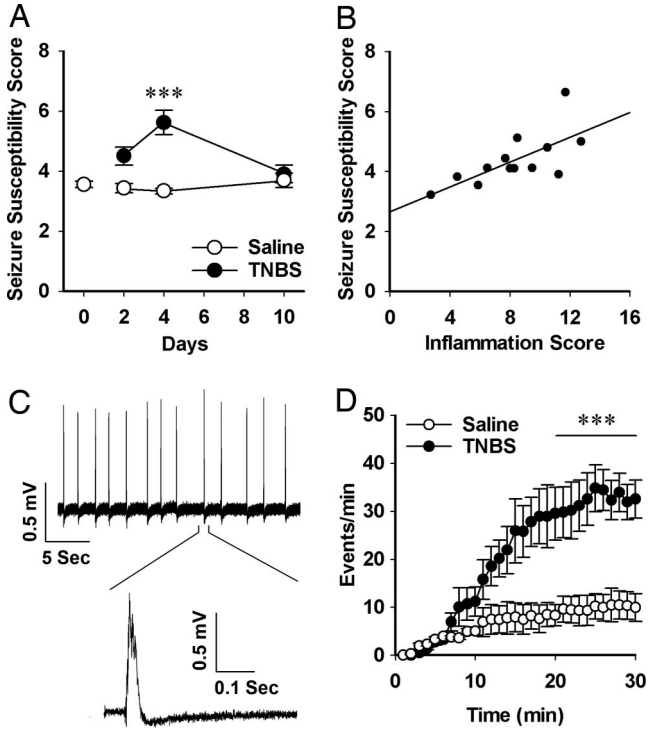

Fig. 1. Peripheral inflammation increases brain excitability. (*A*) PTZ-induced seizure susceptibility before (day 0) and at 2, 4, and 10 days after colonic TNBS or saline injections. Rats that developed TNBS-induced colitis showed significantly greater seizure susceptibility index scores compared with saline-treated controls on day 4 after administration (***, $P < 0.001$; $n = 4$–5 per time point). (*B*) Correlation between the bowel damage score (severity of inflammation) and PTZ-induced seizure susceptibility scores ($n = 13$) in animals receiving TNBS. A significant correlation was observed between the bowel score and seizure susceptibility scores ($r = 0.689$, $P < 0.01$). (*C*) Perfusion of 4-AP (100 μM) on the hippocampal slices caused spontaneous epileptiform activity. Typical interictal activity as recorded in pyramidal cell layer of CA1 hippocampal area is displayed with the expanded area showing the typical appearance of individual interictal bursts with multiple negative-going potentials riding on the larger positive burst. (*D*) A comparison of the frequency (events per minute) of spontaneous interictal activity in the CA1 hippocampal slice over 30 min of perfusion with 4-AP between rats treated 4 days previous with TNBS ($n = 8$) or saline ($n = 7$). Animals with peripheral inflammation show significantly greater number of bursts/minute (***, $P < 0.001$).

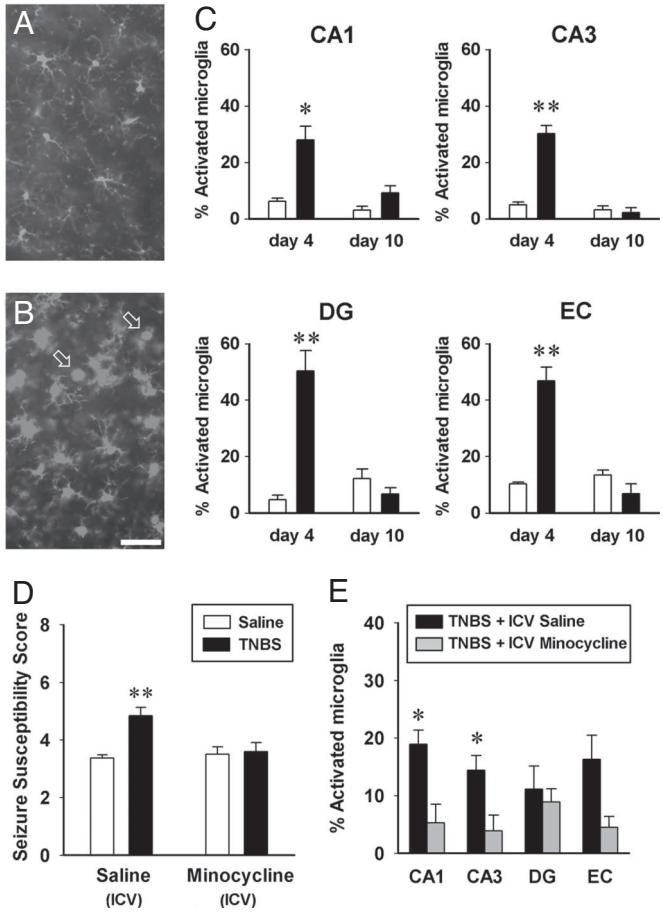

Fig. 2. Increased microglia activation in the hippocampus 4 days after TNBS-induced inflammation. (*A*) Photomicrographs of the CA1 region of the hippocampus from a saline-treated rat that shows many inactive microglia that appear ramified in shape. (*B*) Area CA1 of the hippocampus from an animal treated with TNBS that shows numerous activated microglial cells with retracted processes and perikaryal hypertrophy. (Magnification: ×200; scale bar: 50 μm.) (*C*) Comparison of the percentage of activated microglia from the CA1, CA3, DG, and EC areas between TNBS- (black bars) and saline-treated (white bars) rats (*, $P < 0.05$; **, $P < 0.01$; $n = 3$–4 per group). (*D*) Seizure susceptibility of TNBS- or saline-treated rats after i.c.v. saline (5 μl/day for 4 days) or minocycline (100 μg/day for 4 days) injections (**, $P < 0.01$; $n = 5$–7 per group). (*E*) Percent activated microglia in TNBS-treated rats (day 4) after i.c.v. saline ($n = 3$) or minocycline ($n = 3$). *, $P < 0.05$.

4-aminopyridine (4-AP), an *in vitro* model of epileptogenesis. A recording electrode was placed in the CA1 stratum pyramidale to record spontaneous epileptiform discharges elicited by perfusion of the slice for 30 min with 100 μM 4-AP (19). The mean frequency of the 4-AP-induced interictal discharges was used as an index of slice excitability (20, 21). In slices from both control ($n = 7$) and TNBS-treated ($n = 8$) animals, 4-AP induced spontaneous interictal bursting (Fig. 1*C*). However, the frequency of interictal bursts (events per minute) in TNBS-treated rats was significantly greater when compared with saline-treated controls (32.3 ± 1.4 vs. 9.9 ± 0.9, $t = 20.13$, $P < 0.001$; Fig. 1*D*).

Peripheral Inflammation Increases Microglial Activation. To quantify the severity and extent of CNS inflammation accompanying the peripheral inflammation, we measured microglial activation at 4 and 10 days after treatment. The total number of microglia and the percentage of activated vs. inactive microglia in hippocampal CA1, CA3, dentate gyrus (DG), and entorhinal cortex (EC) were compared between rats treated with TNBS or saline (Figs. 2 *A*–*C*). There was a significantly greater proportion of activated microglia in CA1, CA3, DG, and EC in rats that received TNBS 4 days earlier. By day 10, however, there were no significant differences between the TNBS-treated and control animals in the percentage of activated microglia in any of the four areas.

To test whether the activated microglia play a role in increased CNS excitability, we blocked microglial activation using daily i.c.v. injections of minocycline. Minocycline has marked anti-inflammatory effects on microglia, aside from its antibiotic properties, resulting in a reduction in cytokine expression (22, 23). Because minocycline also acts on peripheral inflammatory processes and therefore is likely to affect the colonic inflammation, we administered it directly into the brain. Minocycline treatment did not affect the seizure susceptibility in saline-treated controls, but in TNBS-treated animals, i.c.v. minocycline treatment significantly decreased the scores to control levels (Fig. 2*D*). To confirm that the minocycline effect was through inhibition of microglial activation, we performed microglial cell quantification by immunohistochemistry in hippocampal areas of TNBS-treated animals. Consistent with our seizure susceptibility results from TNBS-treated rats, chronic i.c.v. infusion of

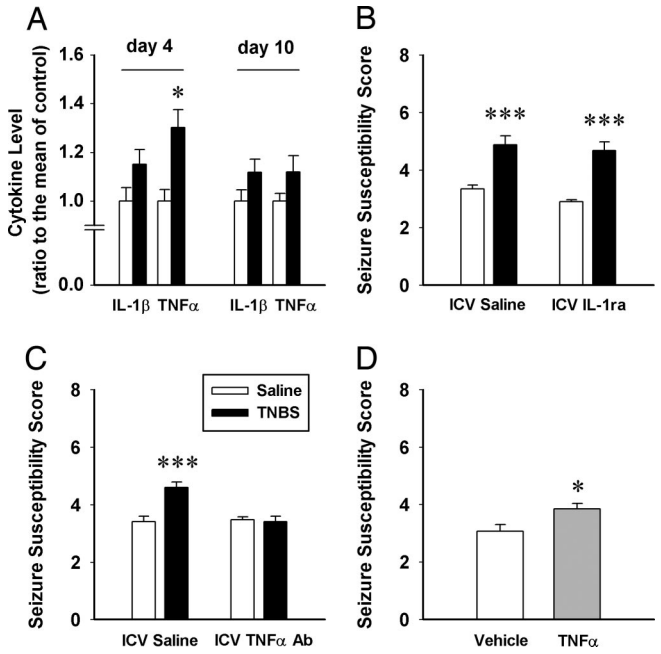

Fig. 3. TNFα mediates the increased CNS excitability during TNBS-induced inflammation. (*A*) Comparison of the levels of IL-1β and TNFα in the hippocampus 4 and 10 days after administration of TNBS or saline. Significantly higher levels of TNFα were present in the TNBS-treated compared with saline-treated rats 4 days after administration (*, $P < 0.01$; $n = 6$ per group). (*B*) The effect of saline (5 μl/day) or IL-1ra (10 μg/day, i.c.v. for 4 days) on the PTZ seizure susceptibility in animals with TNBS-induced inflammation or intracolonic saline administration. IL-1ra injection was ineffective at blocking the seizure-facilitating effects of TNBS inflammation (***, $P < 0.001$; $n = 4–6$ per group). (*C*) Blocking TNFα by i.c.v. administration of the TNFα antibody (10 μg/day, for 4 days), but not by i.c.v. saline (5 μl/day) inhibited the increase in seizure susceptibility observed with TNBS inflammation (**, $P < 0.01$; $n = 5–7$ per group). (*D*) The effect of i.c.v. microinfusion of TNFα (1 μg/24 h at 0.5 μl/h for 4 days) on the PTZ-induced seizure susceptibility scores. Rats given TNFα showed significantly greater seizure susceptibility scores compared with those of vehicle-treated controls (*, $P < 0.05$; $n = 6$ per group).

minocycline decreased the percentage of activated microglia in comparison with vehicle-injected controls (Fig. 2*E*). We observed significant reductions in activated microglia in areas CA1 and CA3, a small but nonsignificant difference in the EC ($P = 0.06$), and no difference in the DG. Inflammation scores from the bowels of TNBS-treated animals were not significantly different between minocycline- and saline-treated controls (11.8 ± 1.0 vs. 12.6 ± 0.6, $P > 0.05$), suggesting that i.c.v. minocyline does not influence the peripheral inflammatory process.

Increases in Central Cytokines Underlie Increased Seizure Susceptibility. Because activated microglia synthesize and release cytokines, we measured the levels of proinflammatory cytokines in the hippocampus at 4 and 10 days after the induction of inflammation. On day 4, we found a small but nonsignificant difference in IL-1β concentration (TNBS, 1214.1 ± 64.2 vs. control, 1055.7 ± 57.3 pg/mg protein, $P = 0.096$); however, in TNBS-treated rats, the level of TNFα was significantly higher than control animals (TNBS, 842.7 ± 30.6 vs. control, 647.7 ± 30.1 pg/mg protein, $P < 0.01$). The levels of both cytokines were no different from control levels 10 days after TNBS treatment (Fig. 3*A*).

Next we determined whether increased levels of central cytokines were responsible for the increased seizure susceptibility by interfering with their action using blockers at doses previously found to be effective (20, 24). Daily i.c.v. injections of IL-1 receptor antagonist (IL-1ra; 10 μg/day) alone for 4 days did not reverse the increased seizure susceptibility in TNBS-treated animals, whereas a TNFα antibody (10 μg/day) inhibited the increased seizure susceptibility in animals treated with TNBS ($P < 0.001$; Figs. 3 *B* and *C*). Vehicle injections were without effect on the inflammation-induced increase in seizure susceptibility. Bowel inflammation scores in TNBS-treated rats showed no significant difference between vehicle- and TNFα antibody-treated rats, ruling out the possibility that i.c.v. administration of TNFα antibody might affect peripheral inflammation (13.6 ± 0.4 vs. 13.5 ± 0.2, $P > 0.05$).

Because interference with the action of TNFα by its neutralizing antibody suggests a role for this cytokine in increased seizure susceptibility, we asked if chronic exposure to TNFα would mimic the effects of peripheral inflammation. We used a concentration of TNFα previously shown to affect CNS function (25, 26). Chronic i.c.v. microinfusion of TNFα (1 μg/24 h at 0.5 μl/h for 4 days) to noninflamed rats resulted in an increase in seizure susceptibility similar to what was seen with bowel inflammation ($P < 0.05$; Fig. 3*D*). This finding provides complementary evidence for the involvement of TNFα in the increase in central excitability following peripheral inflammation.

Discussion

We have shown that inflammation in a peripheral organ, the bowel, is associated with a more excitable CNS, as revealed by a reversible increase in seizure susceptibility that correlates with the severity of the inflammation. Moreover, electrophysiological recordings from hippocampal slices from inflamed animals revealed increased excitability and epileptiform burst discharges to bath application of 4-AP. Augmented TNFα signaling and microglial activation within the brain appear necessary and sufficient for changes in seizure susceptibility.

The induction of seizures by i.v. infusion of PTZ, a $GABA_A$ receptor antagonist, is a standard and sensitive experimental model of clinical myoclonic seizures (27, 28). There are various ways that inflammation of the colon could cause an alteration in PTZ seizure threshold. As seizure generation is highly sensitive to brain temperature (29), it is possible that the peripheral inflammation caused a high fever that made the brain more susceptible to the PTZ. This is unlikely, however, as animals with TNBS colitis do not display fever (15, 30). We next considered the possibilities that altered PTZ metabolism, or an altered blood brain barrier that occurs in this inflammatory model (18), may result in an increased uptake of PTZ within the brain. To address this possibility, we examined *in vitro* excitability of the hippocampus, one of the most seizure-prone structures of the brain. These experiments revealed increased intrinsic excitability of hippocampal neuronal networks after peripheral inflammation as shown by increased spontaneous burst-firing events following application of the potassium channel blocker 4-AP. Our data from the hippocampal slice are in line with previous reports showing enhanced amygdala neuronal excitability *in vitro* after visceral (31) and somatic (32) inflammation. Although 4-AP-induced bursting is known to require alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (33, 34), it is difficult to speculate on a cellular or subcellular target because of the many other effects of 4-AP that contribute to bursting in the isolated hippocampus (35). A known action of 4-AP is to facilitate the release of neurotransmitters such as glutamate, which is one of the factors contributing to the epileptogenic action of 4-AP (33). In some types of experimental peripheral inflammation there are alterations in glutamate receptors (36). Thus, an altered synaptic physiology in response to peripheral inflammation might explain why 4-AP is more effective. The primary impact of our observations is that an isolated hippocampus taken from an animal with intestinal

inflammation is significantly more excitable than one taken from a control animal. Thus, the presence of peripheral inflammation that alters the intrinsic CNS excitability and the increased seizure susceptibility we and others (37–40) have observed *in vivo* cannot be explained by either increased access of the convulsant to the brain, or other alterations in the physiology (temperature, other circulating factors) of the intact animal.

We observed increased microglial activation and number in the hippocampus and adjacent EC in animals with peripheral inflammation. Microglia are known as exquisite sensors of even minor pathological changes in the CNS (41); in addition, they are activated in response to peripheral inflammation (42). In our model of TNBS colitis, the signal that activates brain microglia is not known; previous studies indicate that circulating cytokines (e.g., TNFα and IL-6) are not elevated in acute colitis (15, 16), but other possible signaling mechanisms could include peripheral (vagal) afferents (43) or monocyte infiltration of the CNS (44). Whatever the mechanism, microglial/macrophage activation is necessary for the alteration in CNS excitability that we observed, as interference with their activation with minocycline reversed the CNS changes. Among their other roles, microglia are known to be involved in the communication between brain and systemic immune system, and they produce a number of proinflammatory cytokines and chemokines that are increased in brain following a systemic inflammatory challenge (45, 46). In keeping with this observation, we showed that there were increases in TNFα, but much less of IL-1β in the hippocampus at the height of colitic inflammation. The preferential up-regulation of TNFα relative to IL-1β is interesting, as it contrasts with what is seen after peripheral injection of lipopolysaccharide where transcripts for both cytokines (47) and protein are acutely up-regulated in the hippocampus (24). It is possible that the bowel inflammation model we used, which represents a more long-lasting condition, relative to single lipopolysaccharide injection, is associated with a depletion or downregulation of IL-1β synthesis compared with that for TNFα.

There is now an abundant literature implicating proinflammatory cytokines in seizures and epilepsy (20, 48–50). In light of our evidence of microglial activation, and the knowledge that CNS cytokines are increased in some models of peripheral inflammation (24, 51, 52), we asked if either IL-1β or TNFα contributed to the reduced seizure threshold. Despite that IL-1β is proconvulsant (53) and IL-1ra is anticonvulsant (54), we observed only a small, nonsignificant effect of IL-1ra. Thus, even though a transient increase in IL-1β might have escaped our detection, this finding also argues against a possible up-regulation of the IL-1β receptor as a contributor to the proconvulsant state. In contrast, blocking of central TNFα signaling alone was effective. Because TNFα is implicated in local inflammatory damage in the colon after TNBS (55, 56), and the CNS can affect colonic inflammation (57), we also verified that the reversal of the reduced PTZ seizure threshold was not due to a reduction in the peripheral inflammatory response.

With high hippocampal levels of TNFα occurring after colitic inflammation, and interruption of TNFα signaling reversing the increases in CNS excitability, there was good evidence to conclude that TNFα was responsible for the phenomenon. If so, one might expect that chronic exposure of the brain to TNFα might induce a similar reduction in seizure threshold. Curiously, a role for TNFα in seizure susceptibility has received much less attention than has IL-1β, possibly because one report suggests that TNFα in the mouse hippocampus reduces seizures (58). We found that the chronic infusion of TNFα into the lateral ventricle for 4 days (a time equivalent to that of maximum colitis and seizure susceptibility after TNBS) resulted in a significant reduction in seizure threshold. The magnitude of the change in seizure susceptibility was slightly weaker than what was observed after TNBS-induced inflammation. This is likely related to the incomplete diffusion of exogenous TNFα to areas of the brain that are both distant from the ventricles and involved in seizure generation (including amygdala and entorhinal cortex). Thus, though it is possible that the site of action of TNFα is not only the hippocampus, this finding, along with our findings of increased TNFα in the brain, allows us to conclude that TNFα is produced within the brain during peripheral colonic inflammation, and this TNFα increases seizure susceptibility.

A potential role for TNFα in our studies would be consistent with our observations of increased epileptiform bursting in recordings from hippocampal slices of TNBS-treated animals. TNFα can independently enhance excitatory synaptic strength by increasing membrane trafficking of AMPA receptors in cultured hippocampal pyramidal neurons (2) and reduce the overall inhibitory currents mediated through $GABA_A$ receptors *in vitro* (2, 5). The resulting synaptic scaling is brought about by TNFα released from microglia (3), which we show are increased in number and activational state during colitis. However, there are a multitude of mechanisms by which cellular excitability can be increased, and our future studies will be directed to an analysis of the cellular changes in the brain in our chronically inflamed rats.

In summary, this study shows that an experimental bowel inflammation causes increased CNS excitability. In this respect it is interesting that epidemiological studies indicate that peripheral inflammatory events, including bowel inflammation, occur more frequently in people with epilepsy (11). Though our studies identified increased hippocampal excitability in particular, it is likely that peripheral inflammation may influence excitability in other brain areas, and could account for some of the neurological and behavioral changes associated with inflammatory diseases (1, 51, 52, 59–61).

Materials and Methods

Animals and Drugs. Adult male Sprague-Dawley rats (Charles River Laboratories) weighing 175–200 g at the beginning of the experiments were used in the study. Rats were housed under specified pathogen-free conditions at a constant temperature (20–21 °C) with food and water available *ad libitum*. The light/dark cycle was 12 h light/12 h darkness with light onset at 0700 h local time. All experiments were conducted in accordance with the Canadian Council on Animal Care regulations and approved by the University of Calgary Animal Care Committee.

TNBS, PTZ, 4-AP, minocycline, and other chemicals were obtained from Sigma-Aldrich, IL-1ra (Anakinra) from Amgen, TNFα antibody (Infliximab) from Centocor, and rat recombinant TNFα from PeproTech.

Peripheral Inflammation. Under brief halothane anesthesia, rats received an intraluminal injection of TNBS (0.5 ml, 50 mg/ml, 50% [vol/vol] in ethanol/saline) into the colon (≈8 cm proximal to the anus) via a cannula. Control animals received the same volume of saline. After seizure susceptibility determination, animals were immediately euthanized with an overdose of pentobarbital and the colon was exposed, cut open longitudinally, and macroscopically scored for degree of damage and inflammation according to a scoring system adapted from previous studies (13, 17) and based on features such as adhesions, diarrhea, and degree of ulceration. Colonic damage was assessed independently of knowledge of seizure responses.

PTZ-Induced Seizure Susceptibility Score. For i.v. PTZ infusion, animals were surgically implanted with a jugular catheter under ketamine/xylazine anesthesia 4 days before TNBS administration. PTZ was dissolved in sterile physiological saline to yield a 1% solution. The PTZ-induced seizure was elicited by the slow i.v. infusion of PTZ by a microinfusion pump to an unrestrained rat through the jugular catheter at a constant rate of 0.58 ml/min. PTZ infusion was halted when general clonus was observed, which was characterized by conspicuous forelimb clonus followed by full clonus of the body. To develop an index of seizure susceptibility we used the following formula: [(Animal weight (g) $\times$ 10)/PTZ solution volume infused (μL)]. With this formula, an increased score reflects a lower dose of PTZ and thus enhanced seizure susceptibility.

Hippocampal Excitability. ***Preparation of hippocampal slices.*** Rats were anesthetized with halothane and decapitated 4 days after TNBS or saline treatment.

The brains were immediately placed in ice-cold high-sucrose slicing solution (0–4 °C) containing: NaCl (87 mM), KCl (2.5 mM), NaH_2PO_4 (1.25 mM), $MgCl_2$ (7 mM), $CaCl_2$ (0.5 mM), $NaHCO_3$ (25 mM), glucose (25 mM), and sucrose (20 mM). Horizontal hippocampal slices (400 μm) were cut with a vibrating slicer and immediately transferred to a submerged chamber containing warm (32 °C) recovery artificial cerebrospinal fluid (aCSF): NaCl (126 mM), KCl (2.5 mM), NaH_2PO_4 (1.2 mM), $MgCl_2$ (1.2 mM), $CaCl_2$ (2.4 mM), $NaHCO_3$ (18 mM), glucose (11 mM), and kynurenic acid (1.5 mM). After 45 min the slices were transferred to a second chamber containing aCSF without kynurenic acid for a minimum of 2 h at room temperature before recording. All solutions were continuously bubbled with 5% CO_2/95% O_2 to maintain a pH of 7.4.

Electrophysiological recordings and* in vitro *seizures. Slices were transferred to a submerged recording chamber that was continuously perfused with aCSF at 32 °C. Extracellular field potentials in CA1 stratum pyramidale were recorded with glass micropipettes as previously described (20). Spontaneous epileptiform discharges were elicited by perfusion of the slice for 30 min with 100 μM 4-AP (19).

Microglial Activation. At 4 and 10 days after TNBS or saline treatment, rats were transcardially perfused with cold PBS and cold 4% paraformaldehyde; brains were removed and immersed in paraformaldehyde for 2–3 days. Blocks were then embedded in paraffin, and 10 μm coronal sections were cut, mounted, and immunolabeled for the presence of mononuclear phagocytes (microglia/macrophages). Briefly, sections were incubated in primary Iba1 antibody (overnight; 1:400, rabbit; Wako Chemicals), followed by the secondary antibody (2 h; 1:400, donkey anti-rabbit IgG [CY3]; Jackson Immunoresearch Laboratories). Hippocampal subsections, including CA1, CA3, and DG, and EC, were analyzed for numbers of activated and nonactivated microglia. We analyzed a representative subsection from each animal that covered an area of 700 $\times$ 550 μm of the hippocampus or EC. Activated microglia were distinguished from inactive microglia by the presence of shorter, less-ramified processes and by their perikaryal hypertrophy and ameboid appearance. Analysis was carried out independently by an individual blinded to the treatment of the animals.

Minocycline Treatment. Under ketamine/xylazine anesthesia, rats were placed in a stereotaxic frame, and a 24 G stainless steel guide cannula was implanted 1 mm above the lateral cerebral ventricle and fixed in place with dental acrylic. After 4 days recovery, colonic inflammation was initiated as described herein. Rats with inflammation and their control groups then received daily i.c.v. injections of either minocycline (100 μg/5 μl/day) (62, 63) or saline (5 μl/day), beginning at the time of inflammation induction. PTZ-induced seizure susceptibility was determined on day 4.

Brain Cytokines. To determine the cytokine levels in the hippocampus of rats treated with TNBS, animals were deeply anesthetized and transcardially perfused with cold PBS 4 or 10 days after treatment. Brains were quickly removed and the hippocampi rapidly dissected out on ice, snap frozen in liquid nitrogen, and stored at −80 °C. TNFα and IL-1β ELISA kits (Biosource) were used to assess the hippocampal concentrations of the respective cytokines. The interassay and intraassay variability (represented as coefficient of variation) for IL-1β was 8.7%–9.7% and 6.7%–8.2%, respectively, with a lower limit of detection at $<$3 pg/ml. Interassay and intraassay variability for TNFα were 3.5%–4.3% and 2.6%–8.2%, respectively, with a lower limit of detection at $<$4 pg/ml. Samples were adjusted according to the protein content.

For central administration of cytokine antagonists, rats underwent surgery for i.c.v. guide cannula insertion as described. After 4 days of recovery, intestinal inflammation was induced with TNBS, and daily i.c.v. injections of saline (5 μl/day), IL-1ra (10 μg/5 μl/day), or TNFα antibody (10 μg/5 μl/day) were given, beginning at the time of inflammation induction. PTZ-induced seizure susceptibility scores were determined on day 4. TNFα was administered using miniosmotic pumps (model 1007D, 0.5 μl/h; Alzet) to deliver 1 μg TNFα per 24 h or vehicle i.c.v. over 4 days in two groups of rats (six in each group). The vehicle contained rat albumin (1 mg/ml) to prevent nonspecific bindings, plus trace chlorbutanol to prevent bacterial growth in the solution. After 4 days TNFα infusion, rats underwent PTZ seizure susceptibility score determination and these were compared to vehicle-infused control animals.

Data Analysis. Data are presented as mean $\pm$ SEM and were analyzed using Student's *t* test, two-way or one-way ANOVA, and Tukey's multiple comparison post hoc tests where necessary. A bivariate Pearson correlation analysis was used to assess the relationship between the bowel damage score and seizure susceptibility. Statistical significance was considered when $P < 0.05$.

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